MAJOR ARTICLE

B Cell Memory to 3 *Plasmodium falciparum* Blood-Stage Antigens in a Malaria-Endemic Area

Jeffrey R. Dorfman,[1,2] Philip Bejon,[1,2] Francis M. Ndungu,[1,3] Jean Langhorne,[3] Moses Mosobo Kortok,[1] Brett S. Lowe,[1,2] Tabitha W. Mwangi,[1,2] Thomas N. Williams,[1,2] and Kevin Marsh[1,2]

[1]Kenya Medical Research Institute and Wellcome Trust Research Laboratories, Kilifi, Kenya; [2]Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Headington, Oxford, and [3]National Institute for Medical Research, Medical Research Council, London, United Kingdom

To gain insight into why antibody responses to malarial antigens tend to be short lived, we studied antigen-specific memory B cells from donors in an area where malaria is endemic. We compared antibody and memory B cell responses to tetanus toxoid with those to 3 *Plasmodium falciparum* candidate vaccine antigens: the C-terminal portion of merozoite surface protein 1 ($MSP1_{19}$), apical membrane antigen 1 (AMA1), and the cysteine-rich interdomain region 1α (CIDR1α) of a protein from the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family. These data are the first to be generated on memory B cells in children who are in the process of acquiring antimalarial immunity, and they reveal defects in B cell memory to *P. falciparum* antigens. Compared with the results for tetanus toxoid, more donors who were positive for antibody to AMA1 and CIDR1α were negative for memory B cells. These data imply that some exposures to malaria do not result in the establishment of stable populations of circulating antigen-specific memory B cells, suggesting possible mechanisms for the short-lived nature of many anti-malarial antibody responses.

Antibody responses to blood-stage malarial antigens are often short lived in children and adults in areas where malaria is endemic, as has been demonstrated by longitudinal field studies [1, 2]. In areas where malaria is either endemic or epidemic, the prevalence and/or level of antibodies to merozoite surface protein 1 (MSP1) [3, 4], apical membrane antigen 1 (AMA1) (S. D. Polley and D. J. Conway, personal communication), rhoptry-associated protein 1 [5, 6], and proteins on the surface of infected red blood cells [7] are higher during or soon after infection than at other times. Because most or all people in such areas are exposed to malarial parasites from time to time, this indicates that antibody levels decrease rapidly after infection. Antibodies are a critical part of immunity to malaria [8], and this frequent loss of antibody responses may delay acquisition of immunity.

We used an enzyme-linked immunospot (ELISPOT)–based method [9–14] to assess memory B cell responses to tetanus toxoid and to 3 *Plasmodium falciparum* blood-stage antigens. It is thought that immunity to all 3 of these *P. falciparum* antigens is important for immunity in naturally exposed populations, and all of them are vaccine candidates. $MSP1_{19}$ (the C-terminal portion of MSP1, also called "MSP1 block 17") is directly involved in invasion of red blood cells by the parasite, and its proteolytic processing is required for successful invasion [15]. AMA1 is thought to be involved in the late stages of parasite binding and/or in orientation of the invading parasite relative to red blood cells [16]. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is a highly polymorphic family of large (260–380 kDa) proteins that the parasite expresses on the surface of infected red blood cells. The cysteine-rich interdomain region 1α (CIDR1α) of the Malayan Camp PfEMP1 was also used as an antigen. Antibodies to $MSP1_{19}$ and AMA1 inhibit invasion of red blood cells [15, 17, 18]; immunization with any of these 3 antigens has been shown to be protective in animal

Received 5 October 2004; accepted 13 December 2004; electronically published 13 April 2005.

Financial support: Wellcome Trust.

This study is published with the permission of the director of the Kenya Medical Research Institute.

Reprints or correspondence: Dr. Jeffrey Dorfman, Kenya Medical Research Institute, PO Box 230, Kilifi, Kenya (jeffreydorfman@yahoo.com).

The Journal of Infectious Diseases 2005;191:1623–30

0022-1899/2005/19110-0007$15.00

models [15, 19], and antibodies to $MSP1_{19}$, AMA1, and surface proteins of infected red blood cells (which includes PfEMP1 proteins) have been shown to be associated with protection against clinical disease and/or high parasitemia in field studies [20–24]. Additionally, CIDR1α is thought to be involved in immune dysregulation [25, 26] and the pathogenesis of severe malarial disease [27].

Here, we assessed antibody and memory B cell responses to these 3 *P. falciparum* antigens and compared them with the responses to tetanus toxoid. In Kenya, tetanus toxoid is usually given as a single course of immunization during infancy, with few opportunities for booster immunizations. Little or no boosting by natural exposure to tetanus toxin is expected [28, 29]. Therefore, we expect most immunity to tetanus toxoid in this population to be the result of long-lasting immunity; thus, the pattern of immunity to tetanus toxoid makes for a suitable benchmark against which patterns of immunity to malarial antigens can be compared. We observed that AMA1- and CIDR1α-specific memory B cells are undetectable in a much larger proportion of donors with specific antibody than is so for tetanus toxoid. These and other data suggest that some exposures to malaria do not result in the establishment of stable populations of circulating antigen-specific memory B cells.

DONORS, MATERIALS, AND METHODS

Study population. Venous blood samples were collected from 15 adults (up to 15 mL) and 57 children (up to 5 mL; age range, 5–112 months; median age, 48 months) resident in Ngerenya, Kilifi District, Kenya [30], during April and May 2004, just before the main malaria season. Entomological inoculation rates in the area are generally 1–10 infective bites/person/year [31]. The children involved in the present study were members of a cohort who were being followed by active surveillance for mild episodes of malaria [32]. At the time the samples were collected, all donors were tested for parasitemia by blood slide; very few were positive (6/57 children and 0/15 adults), compared with the observations from previous years [33], presumably because the malaria season before the samples were collected was mild. Ethics approval was granted by the Kenya Medical Research Institute National Ethical Committee. Informed consent was obtained from the adult donors and from the parents or guardians of child donors.

Antigens. Tetanus toxoid was obtained from the National Institute for Biological Standards and Control (Hertfordshire, United Kingdom); AMA1 was a gift from M. Blackman, National Institute for Medical Research (NIMR; London, United Kingdom); $MSP1_{19}$ was a gift from W. Morgan and A. Holder, NIMR; and CIDR1α [34] was expressed as described elsewhere [35]. Keyhole limpet hematocyanin (Sigma) served as a negative control.

Detection of antibody in plasma samples. Antibody levels were determined by ELISA in Falcon Pro-Bind 96-well plates (Becton Dickenson). Wells were coated with antigen and then blocked with PBS containing 0.05% Tween-20 and 2% Marvel nonfat dry milk (PBSTM). Plasma samples, diluted to a concentration of 1:300 in PBSTM, were incubated in duplicate; plates were developed with horseradish peroxidase–conjugated rabbit anti–human IgG (Dako) followed by *o*-phenylene diamine (Sigma). Optical density (OD) was read at 492 nm, and antibody levels are reported as log-transformed OD units. The dilution was chosen on the basis of a titration of known immune and nonimmune serum samples. All ELISAs for a given antigen were performed together.

Detection of memory B cells. The assay was based on previous protocols [10–14]. Peripheral-blood mononuclear cells (PBMCs) were recovered by centrifugation over lymphoprep (Axis-Shield PoC AS) and were precultured in 24-well plates at 8×10^5 cells/well for 5 days (37°C and 5% CO_2) in nutrient-supplemented RPMI 1640 medium with 10% fetal calf serum (FCS; Sigma), 0.5 μg/mL pokeweed mitogen (Sigma), and a 1×10^{-4} dilution of *Staphylococcus aureus* strain Cowan extract (Sigma). ELISPOT plates (Millipore) were precoated with test antigen or rabbit anti–human IgG (Dako) and were blocked with 10% FCS. After preculture, cells were washed and divided between antigen- and anti-IgG–coated wells. After 7–9 h, plates were developed with horseradish peroxidase–conjugated rabbit anti–human IgG (Dako) in PBSTM followed by 3-amino-9-ethylcarbazole (AEC; Sigma) in 50 mmol/L acetate (pH 5.0). Cultures producing <7 IgG spot-forming cells/5000 starting PBMCs rarely appeared to be positive for antigen-specific memory B cells and were therefore rejected. This lead to the exclusion of 5 of the 57 children and 2 of the 15 adults, failure rates similar to those observed previously [36]. Responses to $MSP1_{19}$ were analyzed in samples from 39 children and 5 adults only, and responses to CIDR1α were analyzed in samples from 24 children and 11 adults only.

Calculation of memory B cell frequencies. All cells available from each sample were precultured at a concentration of 8×10^5 cells/well, with a mean of 6.1 wells tested/sample (median, 5; interquartile range, 4–7). A well was measured as positive if any antigen-specific antibody secreting cells (i.e., spot-forming cells) were detected in the ELISPOT assay at the end of the preculture, because it must have been seeded by at least 1 responding memory B cell specific for the antigen in question. To estimate the frequency of memory B cells in the original PBMC sample, we considered the proportion of wells positive for each donor to be an approximation of the true likelihood of positivity for each well. This allowed us to use a poisson distribution calculation to estimate the precursor frequency for the antibody-secreting cells, which is the frequency of memory B cells in the original PBMC sample. Where all wells were positive, it was not possible to fit a poisson distribution to a

100% likelihood of positivity. Instead, we substituted a value as if a half well were negative (for tetanus toxoid, $n = 9$; for AMA1, $n = 1$; and for $MSP1_{19}$ and CIDR1α, $n = 0$). Where all wells were negative, we assigned a precursor frequency of 0. For the purposes of comparison with some previous studies, we also calculated the frequency per 1×10^6 B cells (within PBMCs) [12, 37, 38] and the proportion of IgG-secreting cells that were antigen specific [11–14]. We noted that the estimated frequency per PBMCs correlated well with the proportion of IgG-secreting cells that were antigen specific ($r = 0.758$, $r = 0.855$, $r = 0.936$, and $r = 0.800$, for tetanus toxoid, AMA1, $MSP1_{19}$, and CIDR1α, respectively).

Statistical analysis. Stata (version 8.2; StataCorp) was used for analysis. Antibody positivity was determined as >2 SDs above the mean of OD units for 10 negative donors. For *P. falciparum* antigens, negative donors were unexposed Europeans. For tetanus toxoid, donors were neonates presenting to Kilifi District Hospital with neonatal tetanus and their unimmunized mothers. Data for antibody levels (optical densities) were normalized by log transformation before analysis. Samples classified as negative were assigned one-half the cutoff value for all regression, correlation, and line-fit calculations. In ELISPOT assays, spot-forming cells were counted by use of an ELISPOT plate reader (Autoimmun Diagnostika; version 3.0) and were confirmed by visual inspection. PBMC yield varied, so varying numbers of cultures were analyzed per donor, and donors who had more cultures analyzed were more likely to be positive. We accounted for this when memory B cell frequency was compared with other variables as follows: β coefficients, r values, significance levels, and errors were calculated by linear regression and were adjusted by including the logarithm of the number of wells tested as an additional explanatory variable in the linear regression model. All comparisons of proportions positive or negative were tested for significance by Fisher's exact test; $P < .05$ was considered to be statistically significant.

RESULTS

Estimation of memory B cell frequencies and correlation with antibody levels. It was an open question whether memory B cells would be observed at all. However, memory B cells specific for each *P. falciparum* antigen were detected in some children and adults and at frequencies just below those found for tetanus toxoid (figure 1). There were no significant differences between the children and adults, except that more adults had CIDR1α-specific memory B cells than did children (7/11 [64%] adults vs. 5/24 [21%] children; $P = .019$).

The frequencies of tetanus toxoid–specific memory B cells measured here (figure 1) were similar to previous frequencies of memory B cells specific for tetanus toxoid [38], hepatitis B surface antigen [10], and vaccinia virus (if immunized >1 year

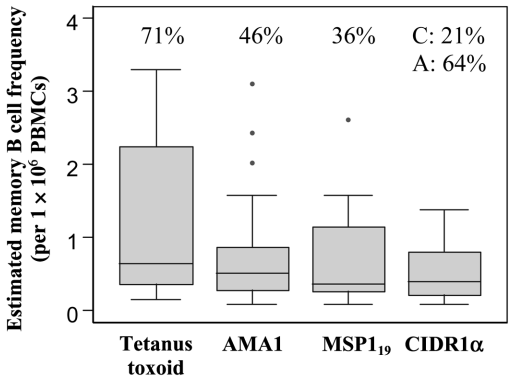

Figure 1. Estimated memory B cell frequencies among positive donors, expressed as frequency per 1×10^6 peripheral-blood mononuclear cells (PBMCs). Data from adults and children are combined. Box plots depict median values, with 25th- and 75th-percentile values represented by the bottom and top edges of boxes. Error bars include all values within 1.5 times the interquartile range of the box. Dots indicate values that fall outside of the error bars. The percentages given above the bars are the percentages of donors who were positive. Only for the cysteine-rich interdomain region 1α (CIDR1α) were the percentages different for adults (A) and children (C). AMA1, apical membrane antigen 1; $MSP1_{19}$, C-terminal portion of merozoite surface protein 1.

before) [13] and yet were lower than frequencies of tetanus toxoid–specific memory B cells measured in other studies [11, 37]. One study found that the frequency of memory B cells specific for tetanus toxoid and diphtheria toxoid increased with the number of previous booster immunizations [11]; low booster rates may explain the lower frequencies of tetanus toxoid–specific memory B cells found here.

Correlations between memory B cell frequencies and serum antibody levels are generally interpreted to suggest that memory B cells give rise to the plasma cells that produce the serum antibody that persists beyond exposure [12, 13]. We found correlations between antibody levels and memory B cell frequencies for all antigens except CIDR1α, for which the trend was present but not significant (figure 2).

Different patterns of immune response for* P. falciparum *antigens than for tetanus toxoid. We wished to understand to what extent there were donors who were negative for memory B cells yet positive for antibody, or the reverse. Responses to tetanus toxoid were used as a model for ideal or near-ideal responsiveness in this population and as a control for any technical difficulties in memory B cell measurement. Among donors who were positive for antibody, the proportion who were negative for memory B cells was significantly higher for AMA1 (49%) and CIDR1α (55%) than for tetanus toxoid (20%) ($P = .001$ and $P = .005$, respectively) (figure 3), indicating a defect in the establishment, maintenance, or circulation of specific memory B cells even after exposure sufficient to induce antibody production. Notably, the same effect was seen when data from only the adults were considered: of 13 adults positive for

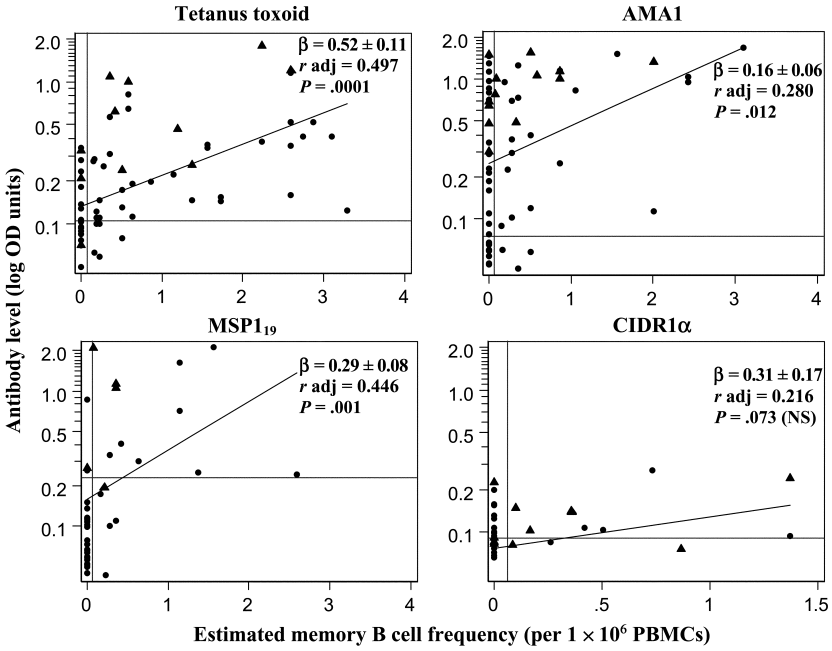

Figure 2. Association between estimated memory B cell frequencies and antibody levels. Circles represent child donors, and triangles represent adult donors. β coefficients, line fits, significance levels, *r* values, and errors were calculated by linear regression after antibody levels classified as negative had been assigned one-half the cutoff value. β coefficients, significance levels, *r* values, and errors for memory B cell frequencies were adjusted as described in Donors, Materials, and Methods. Horizontal lines indicate the cutoff values for antibody levels (>2 SDs above the mean for 10 negative donors); all values for memory B cell frequencies to the left of the vertical lines are zero. AMA1, apical membrane antigen 1; CIDR1α, cysteine-rich interdomain region 1α; MSP1$_{19}$, C-terminal portion of merozoite surface protein 1; NS, not significant.

AMA1 antibody, 5 (38%) were negative for memory B cells; and of 7 adults positive for CIDR1α antibody, 2 (29%) were negative for memory B cells. Thus, adults are not substantially different from children with respect to this effect. A higher proportion of donors who were positive for MSP1$_{19}$-specific memory B cells were negative for antibody (5/16), compared with the proportion of donors who were positive for tetanus toxoid–specific memory B cells yet negative for antibody (5/46) ($P = .07$).

Correlations with age. AMA1 antibody levels correlated with age ($r = 0.566$; $P < .0001$) (figure 4), as has been previously reported [24, 39]. Correlation between age and MSP1$_{19}$ antibody levels has been observed in some studies [40, 41] but not others [42, 43] and was not observed in the present study (data not shown). However, more adults (7/13; 54%) than children (12/52; 23%) were positive for MSP1$_{19}$ antibody in the present study ($P = .036$). The positivity rate for tetanus toxoid antibody ($r = -0.286$; $P = .039$) and the frequency of tetanus toxoid–specific memory B cells ($r = -0.444$; $P < .001$) decreased with age (figure 4), presumably because of waning immunity, as booster immunizations are presumably rare. Although the positivity rate for AMA1 antibody did correlate with age, there was, surprisingly, no association between the frequency of AMA1-specific memory B cells and age ($r = 0.137$; $P = .25$) (figure 4).

No effect associated with current parasitemia or with history of clinical episodes. All children and adults were tested for current parasitemia by microscopic analysis of blood slides; additionally, these children were under active surveillance for clinical episodes of malaria [32]. There were only 6 children positive for parasites by microscopic analysis of blood slides among those analyzed for AMA1-specific memory B cells, 4 among those analyzed for MSP1$_{19}$-specific memory B cells, and 3 among those analyzed for CIDR1α-specific memory B cells—too few to explain the failure to detect memory B cells in so many donors. Additionally, being positive for AMA1 (or CIDR1α) antibody yet negative for memory B cells was not associated with time since last documented clinical episode of malaria or number of clinical episodes during the previous season or the previous 2 seasons (data not shown).

Lack of substantial bias due to variable success of precultures. Variability of B cell growth in vitro in the stimulation precultures might obscure in vivo variation. In such a case, cross-correlations between positivity for memory B cells specific for different antigens would be expected, because positivity for one antigen would be a partial predictor of positiv-

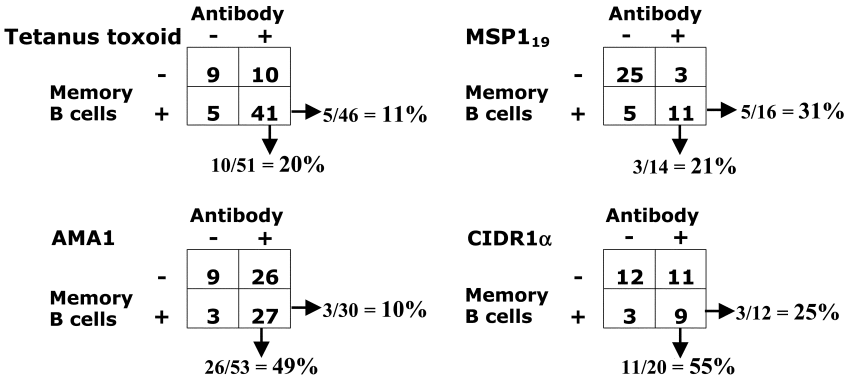

Figure 3. Comparisons of antibody and memory B cell positivity for each antigen. Numbers indicate the number of donors in each category. Percentages indicate either the proportion of donors positive (+) for antibodies yet negative (−) for memory B cells (below) or the proportion of donors positive for memory B cells yet negative for antibodies (right). Data from adults and children were aggregated because they were not different from each other for these proportions. AMA1, apical membrane antigen 1; CIDR1α, cysteine-rich interdomain region 1α; MSP1$_{19}$, C-terminal portion of merozoite surface protein 1.

ity for any other. Positivity for AMA1 and MSP1$_{19}$ correlated ($P = .03$), probably because donors tended to be exposed to both antigens simultaneously. Importantly, positivity for AMA1, MSP1$_{19}$, and CIDR1α did not correlate with tetanus toxoid positivity ($P = .42$, $P = .74$, and $P = .26$, respectively).

The health of the culture, as measured by the number of IgG-producing cells per starting PBMCs, may also skew the results, because unhealthy cultures are less likely to be positive for antigen-specific antibody-secreting cells. Positivity for each antigen was compared with the mean number of IgG-producing cells per well; although there was often a trend toward more positive results as IgG-producing cells increased, this trend never reached statistical significance (for tetanus toxoid, $P = .081$; for AMA1, $P = .16$; for MSP1$_{19}$, $P = .061$; and for CIDR1α, $P = .33$ [χ^2 test for trend of odds]). Taken together, these results suggest that our data were not strongly skewed by the bias of some preculture wells performing better than others.

DISCUSSION

The short-lived nature of many immune responses to *P. falciparum* blood-stage antigens in those acquiring immunity in areas where malaria is endemic may lead one to question whether memory B cells are established at all in such a population. Here, we found memory B cells specific for all 3 antigens tested (figure 1), even in very young children (figure 3 and data not shown) and at frequencies just below those found for tetanus toxoid–specific memory B cells in the same population.

The correlation between memory B cell frequencies and antibody levels has been used by some researchers to test the hypothesis that antibody-secreting plasma cells are drawn from the pool of resting memory B cells, with tetanus toxoid, measles [12], and vaccinia virus [13]. Others, finding no correlation between responses to tetanus toxoid [11, 38] and diphtheria toxoid [11] in human donors, have suggested that circulating antibody is provided by long-lived plasma cells [44], which rarely circulate in peripheral blood [45]. Long-lived plasma cells can have a half-life of a few months in mice [46] and, in the absence of memory B cells, might contribute to sustained antibody production for the lifetime of a mouse (1–2 years); but, it is presumed that memory B cells are required to achieve lifelong antibody immunity in humans [47]. Here, we confirmed the correlation between antibody level and memory B cell frequency for tetanus toxoid, and we also observed correlations between antibody levels and memory B cell frequencies for the 3 *P. falciparum* antigens tested (figure 2). This suggests that B cell memory is a substantial determinant of levels of antibodies to malarial antigens in many individuals.

In Kenya, vaccination with tetanus toxoid is routine during the first 3 months of life; booster vaccination is infrequent. The 10 donors who were positive for antibody to tetanus toxoid but negative for memory B cells were too old to have been recent vaccine recipients who had not yet established memory (the youngest was >3 years old) (figure 4). It seems more likely that some of them had been immunized a long time ago and that their memory B cell frequencies were falling below the detection limit faster than were their antibody levels. Their status as positive for antibody yet negative for memory B cells presumably reflects that, although they no longer had detectable memory B cells, their long-lived plasma cells remained and secreted antibody for some time. It was because of this pattern of immunity, which is relatively easy to explain, that responses to tetanus toxoid were used as a model for ideal or near-ideal responsiveness in this population and as a control for any technical difficulties in memory B cell measurement.

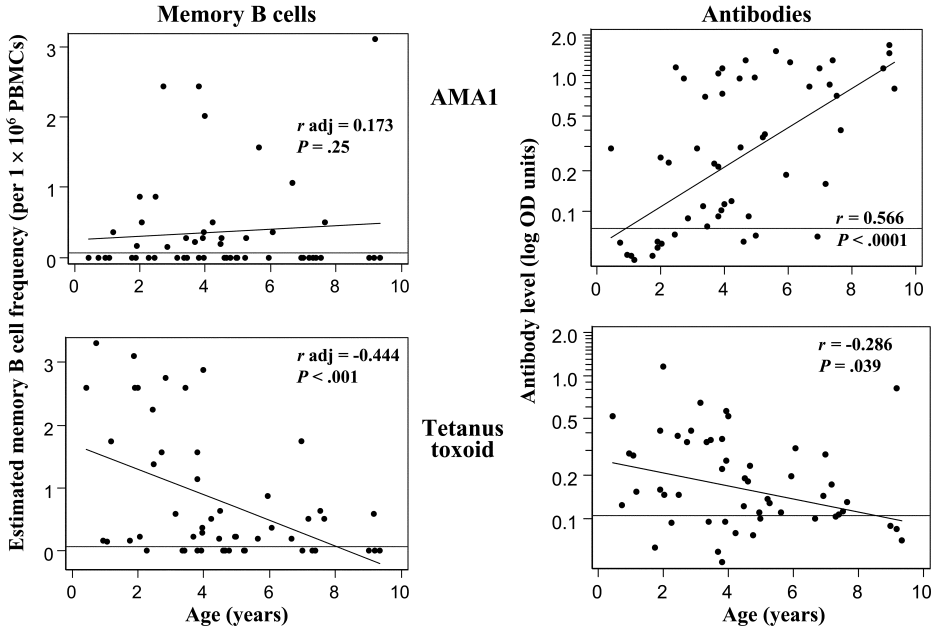

Figure 4. Changes with age of children in levels of antibody and estimated frequencies of memory B cells specific for apical membrane antigen 1 (AMA1) and tetanus toxoid. Line fits, significance levels, *r* values, and errors were calculated by linear regression after antibody levels classified as negative were assigned one-half the cutoff value. Significance levels, *r* values, and errors for memory B cell frequencies were adjusted as described in Donors, Materials, and Methods. Horizontal lines indicate the cutoff values (>2 SDs above the mean for 10 negative donors) for antibody levels; for memory B cell frequencies, all values below the horizontal lines are zero.

More donors who were positive for $MSP1_{19}$-specific memory B cells were negative for antibody (31%), compared with the proportion for tetanus toxoid (11%) ($P = .07$) (figure 3). In some individuals, hepatitis B antibody levels wane during the years after immunization, and yet memory B cells persist [9, 10, 48]; these individuals are protected from disease and develop anamnestic antibody responses on revaccination [48]. The persistence of $MSP1_{19}$-specific memory B cells (which needs to be confirmed in subsequent studies) may result in a greater ability to quickly produce new antibody on reinfection.

Importantly, more donors who did not have detectable circulating memory B cells specific for AMA1 (49%) and for CIDR1α (55%) developed antibody responses than did those who did not have detectable memory B cells specific for tetanus toxoid (20%) ($P = .001$ and $P = .005$, respectively) (figure 3). AMA1 and CIDR1α were tested simultaneously with tetanus toxoid, and we can identify no technical reason to explain this difference. In fact, we suggest above that at least some of the donors who were positive for antibody yet negative for memory B cells were so because their memory B cells were waning as a result of a lack of exposure, which should not apply to *P. falciparum* antigens in an area endemic for malaria. Notably, some of the donors who were positive for antibody yet negative for memory B cells were adults (for AMA1, 5/13; for CIDR1α, 2/7); although the number of adults analyzed here was small, this finding suggests that these memory B cell defects may persist even in apparently immune adults.

A previous study in adults found a relationship between memory B cell frequencies and serum antibody levels for *P. falciparum* extract [49] (as we did here for our antigens [figure 2]) yet noted only 1 donor among 18 adults who was positive for antibody yet negative for memory B cells. It seems likely that use of a complex mixture of *P. falciparum* proteins in the extract may have obscured the effect we observed here, because the donors who were negative for memory B cells specific for one antigen in the extract were, presumably, still likely to be positive for memory B cells specific for another antigen in the extract. Other studies have investigated the association between memory B cell positivity and serum antibody positivity for 2 peptides derived from 1 *P. falciparum* blood-stage antigen [50] and for a selection of recombinant blood-stage antigens [36]. The sample sizes in both of these studies were small ($n = 15$, for each), however, and no comparisons to a nonmalarial antigen were made; thus, analyses similar to ours are not possible with those data.

Surprisingly, despite the correlation between age and AMA1 antibody levels, there was no such correlation between AMA1-specific memory B cell frequency and age (figure 4). One plausible explanation for this phenomenon and for the presence of so many donors positive for antibody yet negative for memory

B cells is that AMA1-specific memory B cells are specifically retained in lymphoid tissues and do not circulate in some donors, a phenomenon for which there is precedent [51]. This retention occurs with T cells during clinical episodes of malaria [52]; thus, current or recent parasitization would be one of the more likely causes of memory B cell retention. However, of those analyzed for AMA1-specific memory B cells, only 6 children were positive for parasites, and of those analyzed for $MSP1_{19}$- or CIDR1α-specific memory B cells, even fewer were positive—too few to explain possible B cell retention in our population. Additionally, being positive for AMA1 (or CIDR1α) antibody yet negative for memory B cells was not associated with time since last documented clinical episode of malaria or number of clinical episodes during the previous season or the previous 2 seasons (data not shown). Although it is difficult to rule out the possibility that recent or current low-level asymptomatic parasitization results in specific retention of AMA1- or CIDR1α-specific memory B cells in lymphoid tissues, the data at hand do not support this explanation. An alternative explanation is that repeated exposures do not result in long-lasting B cell memory. Perhaps repeated stimulation by malarial antigens sometimes results in all specific memory B cells being converted into effector cells, or perhaps the polyclonal activation of B cells by CIDR1α domains [26] could compete with the maintenance of specific memory B cells or inhibit class switching to IgG, as polyclonal B cell activation during viral infection in a mouse model would suggest [53]. Our data do not distinguish between these possibilities well; nonetheless, they reveal evidence that is suggestive of defects in memory B cell populations that warrant further study.

Acknowledgments

We thank the blood donors and people of Ngerenya, for their assistance; Shee Bwetta, Hussein Kivuko, John Bushudi, Samuel Geji, and Augustus Kea, for blood sampling; Bill Morgan and Tony Holder, for provision of $MSP1_{19}$; Mike Blackman, for provision of AMA1; Rafi Ahmed, Nick Willcox, Britta Urban, and Chris Newbold, for advice in assay development; and Robins Stephens and Ariel Achtman, for critical review of the manuscript.

References

1. Cavanagh DR, Elhassan IM, Roper C, et al. A longitudinal study of type-specific antibody responses to *Plasmodium falciparum* merozoite surface protein-1 in an area of unstable malaria in Sudan. J Immunol **1998**; 161:347–59.
2. Giha HA, Staalsoe T, Dodoo D, et al. Nine-year longitudinal study of antibodies to variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes. Infect Immun **1999**; 67:4092–8.
3. al-Yaman F, Genton B, Kramer KJ, et al. Acquired antibody levels to *Plasmodium falciparum* merozoite surface antigen 1 in residents of a highly endemic area of Papua New Guinea. Trans R Soc Trop Med Hyg **1995**; 89:555–9.
4. Soares IS, Oliveira SG, Souza JM, Rodrigues MM. Antibody response to the N and C-terminal regions of the *Plasmodium vivax* Merozoite Surface Protein 1 in individuals living in an area of exclusive transmission of *P. vivax* malaria in the north of Brazil. Acta Trop **1999**; 72: 13–24.
5. Jakobsen PH, Kurtzhals JA, Riley EM, et al. Antibody responses to Rhoptry-Associated Protein-1 (RAP-1) of *Plasmodium falciparum* parasites in humans from areas of different malaria endemicity. Parasite Immunol **1997**; 19:387–93.
6. Fonjungo PN, Elhassan IM, Cavanagh DR, et al. A longitudinal study of human antibody responses to *Plasmodium falciparum* rhoptry-associated protein 1 in a region of seasonal and unstable malaria transmission. Infect Immun **1999**; 67:2975–85.
7. Bull PC, Lowe BS, Kaleli N, et al. *Plasmodium falciparum* infections are associated with agglutinating antibodies to parasite-infected erythrocyte surface antigens among healthy Kenyan children. J Infect Dis **2002**; 185:1688–91.
8. Wipasa J, Elliott S, Xu H, Good MF. Immunity to asexual blood stage malaria and vaccine approaches. Immunol Cell Biol **2002**; 80:401–14.
9. van Hattum J, Maikoe T, Poel J, De Gast GC. In vitro anti-HBs production by individual B cells of responders for hepatitis B vaccine who subsequently lost antibody. In: Margolis H, ed. Viral hepatitis and liver disease. Baltimore, MD: Williams & Wilkins, **1990**:774–6.
10. Wismans PJ, van Hattum J, De Gast GC, et al. The spot-ELISA: a sensitive in vitro method to study the immune response to hepatitis B surface antigen. Clin Exp Immunol **1989**; 78:75–9.
11. Nanan R, Heinrich D, Frosch M, Kreth HW. Acute and long-term effects of booster immunisation on frequencies of antigen-specific memory B-lymphocytes. Vaccine **2001**; 20:498–504.
12. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. Science **2002**; 298:2199–202.
13. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R. Cutting edge: long-term B cell memory in humans after smallpox vaccination. J Immunol **2003**; 171:4969–73.
14. Crotty S, Aubert RD, Glidewell J, Ahmed R. Tracking human antigen-specific memory B cells: a sensitive and generalized ELISPOT system. J Immunol Methods **2004**; 286:111–22.
15. Berzins K. Merozoite antigens involved in invasion. In: Perlmann P, Troye-Blumberg M, eds. Malaria immunology. Vol. 80. Basel: S. Karger AG, **2002**:125–43.
16. Mitchell GH, Thomas AW, Margos G, Dluzewski AR, Bannister LH. Apical membrane antigen 1, a major malaria vaccine candidate, mediates the close attachment of invasive merozoites to host red blood cells. Infect Immun **2004**; 72:154–8.
17. Hodder AN, Crewther PE, Anders RF. Specificity of the protective antibody response to apical membrane antigen 1. Infect Immun **2001**; 69:3286–94.
18. Kocken CH, Withers-Martinez C, Dubbeld MA, et al. High-level expression of the malaria blood-stage vaccine candidate *Plasmodium falciparum* apical membrane antigen 1 and induction of antibodies that inhibit erythrocyte invasion. Infect Immun **2002**; 70:4471–6.
19. Baruch DI, Gamain B, Barnwell JW, et al. Immunization of *Aotus* monkeys with a functional domain of the *Plasmodium falciparum* variant antigen induces protection against a lethal parasite line. Proc Natl Acad Sci USA **2002**; 99:3860–5.
20. Riley EM, Allen SJ, Wheeler JG, et al. Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced malaria morbidity. Parasite Immunol **1992**; 14:321–37.
21. Egan AF, Morris J, Barnish G, et al. Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. J Infect Dis **1996**; 173:765–9.
22. Conway DJ, Cavanagh DR, Tanabe K, et al. A principal target of human immunity to malaria identified by molecular population genetic and immunological analyses. Nat Med **2000**; 6:689–92.
23. Bull PC, Lowe BS, Kortok M, Molyneux CS, Newbold CI, Marsh K.

Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. Nat Med **1998**; 4:358–60.
24. Polley SD, Mwangi T, Kocken CH, et al. Human antibodies to recombinant protein constructs of *Plasmodium falciparum* Apical Membrane Antigen 1 (AMA1) and their associations with protection from malaria. Vaccine **2004**; 23:718–28.
25. Urban BC, Ferguson DJ, Pain A, et al. *Plasmodium falciparum*-infected erythrocytes modulate the maturation of dendritic cells. Nature **1999**; 400:73–7.
26. Donati D, Zhang LP, Chen Q, et al. Identification of a polyclonal B-cell activator in *Plasmodium falciparum.* Infect Immun **2004**; 72:5412–8.
27. Beeson JG, Brown GV. Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. Cell Mol Life Sci **2002**; 59:258–71.
28. Galazka AM. Immunological basis for immunization series. Module 3: tetanus. Geneva: World Health Organization Global Programme for Vaccines and Immunization, Expanded Programme on Immunization, **1993**.
29. Wassilak SGF, Orenstein WA, Sutter RW. Tetanus toxoid. In: Plotkin SA, Mortimer EA, eds. Vaccines. Philadelphia: WB Saunders, **1994**: 57–90.
30. Mwangi TW, Ross A, Marsh K, Snow RW. The effects of untreated bednets on malaria infection and morbidity on the Kenyan coast. Trans R Soc Trop Med Hyg **2003**; 97:369–72.
31. Mbogo CN, Snow RW, Khamala CP, et al. Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. Am J Trop Med Hyg **1995**; 52:201–6.
32. Nyakeriga AM, Troye-Blomberg M, Dorfman JR, et al. Iron deficiency and malaria among children living on the coast of Kenya. J Infect Dis **2004**; 190:439–47.
33. Mwangi T. Clinical epidemiology of malaria under differing levels of transmission [PhD dissertation]. Oxford: Open University, **2003**.
34. Baruch DI, Pasloske BL, Singh HB, et al. Cloning the *P. falciparum* gene encoding PfEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. Cell **1995**; 82:77–87.
35. Allsopp CE, Sanni LA, Reubsaet L, et al. CD4 T cell responses to a variant antigen of the malaria parasite *Plasmodium falciparum*, erythrocyte membrane protein-1, in individuals living in malaria-endemic areas. J Infect Dis **2002**; 185:812–9.
36. Garraud O, Perraut R, Diouf A, et al. Regulation of antigen-specific immunoglobulin G subclasses in response to conserved and polymorphic *Plasmodium falciparum* antigens in an in vitro model. Infect Immun **2002**; 70:2820–7.
37. Lanzavecchia A, Parodi B, Celada F. Activation of human B lymphocytes: frequency of antigen-specific B cells triggered by alloreactive or by antigen-specific T cell clones. Eur J Immunol **1983**; 13:733–8.
38. Leyendeckers H, Odendahl M, Lohndorf A, et al. Correlation analysis between frequencies of circulating antigen-specific IgG-bearing memory B cells and serum titers of antigen-specific IgG. Eur J Immunol **1999**; 29:1406–17.
39. Thomas AW, Trape JF, Rogier C, Goncalves A, Rosario VE, Narum DL. High prevalence of natural antibodies against *Plasmodium falciparum* 83-kilodalton apical membrane antigen (PF83/AMA-1) as detected by capture-enzyme-linked immunosorbent assay using full-length baculovirus recombinant PF83/AMA-1. Am J Trop Med Hyg **1994**; 51: 730–40.
40. Egan AF, Chappel JA, Burghaus PA, et al. Serum antibodies from malaria-exposed people recognize conserved epitopes formed by the two epidermal growth factor motifs of MSP1(19), the carboxy-terminal fragment of the major merozoite surface protein of *Plasmodium falciparum.* Infect Immun **1995**; 63:456–66.
41. Shi YP, Sayed U, Qari SH, et al. Natural immune response to the C-terminal 19-kilodalton domain of *Plasmodium falciparum* merozoite surface protein 1. Infect Immun **1996**; 64:2716–23.
42. Dodoo D, Theander TG, Kurtzhals JA, et al. Levels of antibody to conserved parts of *Plasmodium falciparum* merozoite surface protein 1 in Ghanaian children are not associated with protection from clinical malaria. Infect Immun **1999**; 67:2131–7.
43. John CC, O'Donnell RA, Sumba PO, et al. Evidence that invasion-inhibitory antibodies specific for the 19-kDa fragment of merozoite surface protein-1 (MSP-1 19) can play a protective role against blood-stage *Plasmodium falciparum* infection in individuals in a malaria endemic area of Africa. J Immunol **2004**; 173:666–72.
44. Manz RA, Radbruch A. Plasma cells for a lifetime? Eur J Immunol **2002**; 32:923–7.
45. Slifka MK, Matloubian M, Ahmed R. Bone marrow is a major site of long-term antibody production after acute viral infection. J Virol **1995**; 69:1895–902.
46. Slifka MK, Ahmed R. Long-lived plasma cells: a mechanism for maintaining persistent antibody production. Curr Opin Immunol **1998**; 10: 252–8.
47. Crotty S, Ahmed R. Immunological memory in humans. Semin Immunol **2004**; 16:197–203.
48. West DJ, Calandra GB. Vaccine induced immunologic memory for hepatitis B surface antigen: implications for policy on booster vaccination. Vaccine **1996**; 14:1019–27.
49. Migot F, Chougnet C, Henzel D, et al. Anti-malaria antibody-producing B cell frequencies in adults after a *Plasmodium falciparum* outbreak in Madagascar. Clin Exp Immunol **1995**; 102:529–34.
50. Fievet N, Chougnet C, Dubois B, Deloron P. Quantification of antibody-secreting lymphocytes that react with Pf155/RESA from *Plasmodium falciparum:* an ELISPOT assay for field studies. Clin Exp Immunol **1993**; 91:63–7.
51. Callard RE, McCaughan GW, Babbage J, Souhami RL. Specific in vitro antibody responses by human blood lymphocytes: apparent nonresponsiveness of PBL is due to a lack of recirculating memory B cells. J Immunol **1982**; 129:153–6.
52. Hviid L, Kurtzhals JA, Goka BQ, Oliver-Commey JO, Nkrumah FK, Theander TG. Rapid reemergence of T cells into peripheral circulation following treatment of severe and uncomplicated *Plasmodium falciparum* malaria. Infect Immun **1997**; 65:4090–3.
53. Recher M, Lang KS, Hunziker L, et al. Deliberate removal of T cell help improves virus-neutralizing antibody production. Nat Immunol **2004**; 5:934–42.